

## Fungicide Exposure and Amyloid Plaques in Mice: Further Evidence of an Environmental Risk Factor for Alzheimer's Disease

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For more than a decade, researchers have recognized pesticide exposure as a risk factor for Parkinson's disease.<sup>1</sup> However, these and other environmental risk factors have received limited attention in the study of Alzheimer's disease (AD),<sup>2,3</sup> despite shared disease features. Now, an analysis published in *Environmental Health Perspectives*<sup>4</sup> reports that chronic exposure to very low doses of certain fungicides promoted pathological changes in a transgenic mouse model of AD.

For the new study, weaned mice consumed water containing a mixture of 0.1 µg/L each of cyprodinil, mepanipyrin, and pyrimethanil, three widely used fungicides in the anilinopyrimidine class. "We were interested in these particular fungicides because of their chemical similarity to thienyl pyrimidine, which is derived from  $\alpha$ -terthienyl, a botanical pesticide we had studied before," says Véronique Perrier, a professor of neuroscience at the University of Montpellier in France and the study's senior author. "Since thienyl pyrimidine promotes protein aggregation in prion diseases,<sup>5</sup> we wondered if related compounds may trigger similar processes in Alzheimer's disease."

In addition to drinking water, residues of the three chemicals have been detected in samples of fruits, vegetables, and drinks;<sup>6,7</sup> dust;<sup>8</sup> and air.<sup>9</sup> The European Union has set a drinking water limit of 0.1 µg/L for individual pesticides (such as any one of these fungicides) or 0.5 µg/L for a pesticide mixture.<sup>10</sup> However, the fungicides are not among the pesticides regulated in drinking water by the U.S. Environmental Protection Agency.<sup>11</sup>

Perrier and colleagues used transgenic mice engineered to overexpress human  $\beta$ -amyloid peptides.<sup>12</sup> In the human brain, these peptides aggregate into amyloid plaques, a hallmark of AD. The researchers studied transgenic fungicide-exposed and control mice and similar numbers of exposed and control wild-type mice. After 9 months of exposure, the researchers analyzed all mouse

brains with multiple immunohistochemistry and biochemical assays.

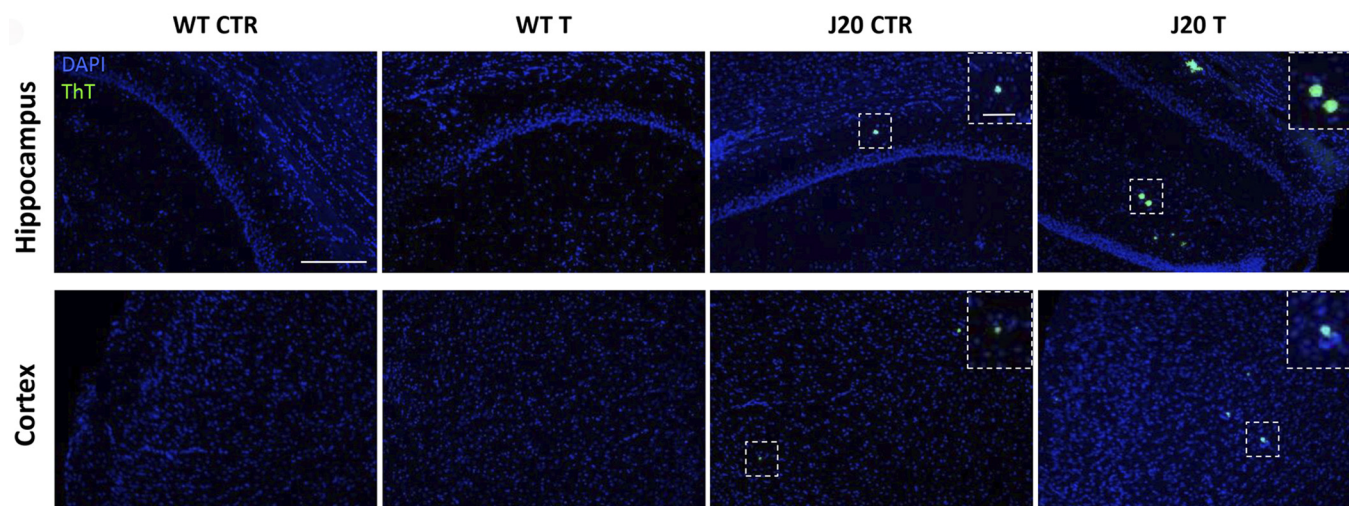
In a longitudinal *in vivo* experiment, the researchers used two-photon microscopy to assess the development of amyloid plaques in the brains of eight transgenic mice (5 exposed, 3 control) after 3, 6, and 9 months of exposure. They also measured the interaction of fungicides with amyloid plaques in brain tissue.

The experiment yielded consistent evidence that chronic exposure to the fungicide cocktail accelerated AD pathology. Compared with control animals, exposed mice had more amyloid plaques, larger plaque volumes, and a greater neuroinflammatory response as measured by activated microglia and astrocytes. Inflammatory processes are believed to contribute to neuronal death and AD progression.<sup>13</sup>

The researchers suggested a potential mechanism for the faster plaque formation in transgenic mice: The fungicide cocktail increased the production of  $\beta$ -site APP cleaving enzyme 1 and decreased the production of neprilysin. Together, this may promote the accumulation and aggregation of  $\beta$ -amyloid peptides into amyloid plaques. The plaques were also found in the blood vessel walls of the transgenic mice. According to the researchers, these stiffened vessel walls may have compromised the blood–brain barrier,<sup>14</sup> allowing the fungicides to reach the brain.

Beate Ritz, a professor of epidemiology at the University of California, Los Angeles, was impressed with the comprehensive nature of the work. "I think the researchers beautifully showed how the mixture of fungicides might affect multiple steps in the process that gives rise to Alzheimer's disease," says Ritz, who was not involved in the study.

Ritz has observed a stronger association between pesticides and Parkinson's disease in genetically susceptible individuals in her own studies.<sup>15,16</sup> She noticed a similar finding for a subgroup



Investigators exposed mice to residues of three fungicides for 9 months, comparable to a 20-year exposure in humans. Exposed J20 mice—a transgenic model that overexpresses human  $\beta$ -amyloid peptides—developed more and larger amyloid plaques (green) in the hippocampus and cortex, compared with wild-type and control J20 mice. Note: WT, wild-type mice; CTR, control; T, treated with fungicide mixture; DAPI, 2-(4-amidinophenyl)-1H-indole-6-carboxamide (a stain for DNA); ThT, thioflavin T (a fluorescent stain for amyloid plaques). Image: Lafon et al. (2020).<sup>4</sup>

of high responders in the transgenic mice. In these susceptible animals, the fungicide exposure generated especially large amyloid plaques in both blood vessels and functional brain tissue.

For Mark Zylka, a professor of cell biology and physiology at the University of North Carolina at Chapel Hill, the new study adds to the intriguing yet limited body of evidence linking fungicides and AD. Zylka's group showed that strobilurins, a separate class of fungicides from the anilinopyrimidines, produced transcriptional changes *in vitro* that mimicked neurodegenerative and neuroinflammatory processes.<sup>17</sup>

"Despite the chemical differences, I think testing the effect of strobilurins in an AD mouse model would be a worthwhile follow-up effort," says Zylka, who also was not involved in the study. Within this group of fungicides, azoxystrobin would be of particular interest, he adds, due to its high prevalence in household dust and mold-resistant wallboard samples.<sup>18</sup>

Another important next step will be to test the three anilino-pyrimidine fungicides in other AD mouse models, says Zylka. This could include the recently proposed AD-BXD panel, which combines high-risk human mutations with a genetically diverse background to better simulate human AD.<sup>19</sup>

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